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Efficacy of *Trichoderma* spp. against anthracnose of chilli (*Capsicum annuum* L.)

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Abstract

To evaluate efficacy of the native isolates of *Trichoderma* species which promote the growth of chilli and to manage anthracnose disease under *in vitro* and field conditions. The dominant pathogen, which causes anthracnose of chilli is *Colletotrichum capsici*. Four native *Trichoderma* antagonists were isolated from rhizosphere soils of different healthy crops like Black gram, Green gram, Ground nut and Chilli from SHUATS, Central Research Farm Allahabad. Under *in vitro* conditions, the results revealed that *Trichoderma viride* isolate 1 was found to be effectively inhibit the radial mycelial growth of the pathogen (by 76.45%) when compared to all other isolates. Under field conditions, the minimum disease intensity (%) was recorded in *Trichoderma viride* isolate 1 + *Trichoderma harzianum* isolate 2 which was applied combinely as seed + soil treatment (36.33%).

Keywords: Chilli, *Colletotrichum capsici*, *Trichoderma viride*, *Trichoderma harzianum*

1. Introduction

Chilli (*Capsicum annuum* L.) is an important vegetable as well as spice crop, cultivated worldwide. It is not only used in many cuisines but also found to have many medicinal properties. Anthracnose is a major problem on mature leaves and fruits, causing severe losses due to both pre-harvest and post-harvest fruit decay^[1]. Biological control is a promising tool to maintain current level of agricultural production while reducing the release of polluting chemical pesticides to the environment. The main mechanism involved in bio-control are antibiosis, myco parasitism and competition for food. *Trichoderma* is free living asexually reproducing and filamentous fungi which act as a myco parasite, avirulent plant symbionts, prolific producers of spores and powerful antibiotics, anti-fungal compounds and secondary metabolites.

2. Material and Methods

2.1 Isolation of *Trichoderma* spp. from rhizosphere soil of different crops

Total four isolates of *Trichoderma* spp. were isolated from crops like Black gram, Green gram, Ground nut, Chilli. Isolation was done by employing serial dilution technique^[2]. The isolates were purified by single spore culture technique. Whitish green to green pigmented fungal colonies identical to *Trichoderma* were maintained on PDA and were used for further study.

2.2 *In vitro* effect of *Trichoderma* antagonists against pathogen

The antagonistic potential of *Trichoderma* isolates was assessed against *Colletotrichum capsici* by dual culture technique on Potato Dextrose Agar (PDA) medium as procedure described^[3]. Five mm disc of fifteen days old fungal cultures were placed on PDA medium one cm away from the edge of the plate, separately. *Trichoderma* spp. (5 mm disc) was placed at opposite side of the Petri plate. Three replicated plates for each treatment was maintained and incubated at 25±1° C. Observation on Per cent inhibition and Colony radius of the pathogen and *Trichoderma* were recorded after 6 and 9 days of inoculation. Per cent inhibition over control was calculated as per the formulae Eq. (1) by Vincent, (1947).

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent inhibition over control C = Growth of test pathogen with absence of antagonist (mm) T = Growth of test pathogen with antagonist (mm)

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2.3 Talc based formulation of *Trichoderma* spp. for seed and soil application in field conditions

A field trial was conducted to check the efficacy of *Trichoderma* isolates using Randomized Block Design with a plot size of 4 m² and 8 treatments with three replications each. The treatment details are given in Table 1. Talc based formulation of *Trichoderma* spp. is been used as a carrier for the seed treatment of chilli crop in nursery conditions and soil

treatment of chilli crop in field conditions. This formulation contained 2.8 x 10⁸ CFU/g of powder. The treated seeds are been kept for 30 minutes and sowed. For field application 10 - 25 g of talc-based powder in 100 m² has been taken treatment wise and mixed before the sowing of the chilli crop [4]. The disease intensity was recorded after first appearance of the disease at regular intervals using 1-9 scale.

Table 1: Treatment details

Treat. No.	Treatment Name
T ₀	Control
T ₁	<i>T. viride</i> @ 4g/kg. Seed Treatment.
T ₂	<i>T. viride</i> @ 4g/kg Seed + Soil Treatment. @ 5kg/ha
T ₃	<i>T.harzianum</i> @ 8g/kg Seed Treatment.
T ₄	<i>T.harzianum</i> @ 8g/kg Seed +soil treatment @ 5kg/ha
T ₅	<i>T.viride</i> @ 4g/kg+ <i>T.harzianum</i> @ 8g/kg. Seed Treatment.
T ₆	<i>T.viride</i> @ 4 kg/ha+ <i>T.harzianum</i> @ 6g/kg. Seed + Soil Treatment@5 kg/ha.
T ₇	Carboxin 37.5% + Thiram 37.5% (Vitavax powder 50 WP) @ 0.1% Foliar Spray.

3. Results and Discussion

3.1 Isolation of *Trichoderma* spp. and Identification by morphological characters

The native population of *Trichoderma* sp. from different habitats was quantified and characterized [5]. Morphological characters of *Trichoderma* spp. with respect to radial growth and colony characters were studied on PDA. Isolation was done by employing serial dilution technique. The isolates were purified by single spore culture technique. The radial growth (mm) of all isolates was measured at 7th day after inoculation. Whitish green to green pigmented fungal colonies identical to *Trichoderma* were maintained on PDA and were

used for further study. The results are presented in Table 2 and Figure 1.

Among four isolates of *Trichoderma* spp. Tr-1, and Tr-3 are milky white to dark green in colour with white yellow and amber colour pigmentation, respectively having sub aerial and disperse mycelial growth. The isolate Tr- 3 and Tr-4 had sub aerial mycelial growth, milky white to grayish green colony colour with yellow pigmentation. The isolated antagonists were identified as *T. viride* isolate 1 (Tr-1), *T. harzianum* isolate 2 (Tr-2), *T. viride* isolate 3 (Tr-3), *T. harzianum* isolate 4 (Tr-4).

Table 2: Morphological characteristics of *Trichoderma* spp.

Sr. No.	Isolates	Radial growth (mm) at 7 DAI	Colony characters	
			Colony growth type	Colony colour
1.	Tr - 1	90.00	Sub aerial and disperse	Milky white to green
2.	Tr - 2	90.00	Sub aerial and disperse	Milky white to grayish green
3.	Tr - 3	90.00	Sub aerial and disperse	Milky white to dark green
4.	Tr - 4	90.00	Sub aerial and disperse	Milky white to grayish green

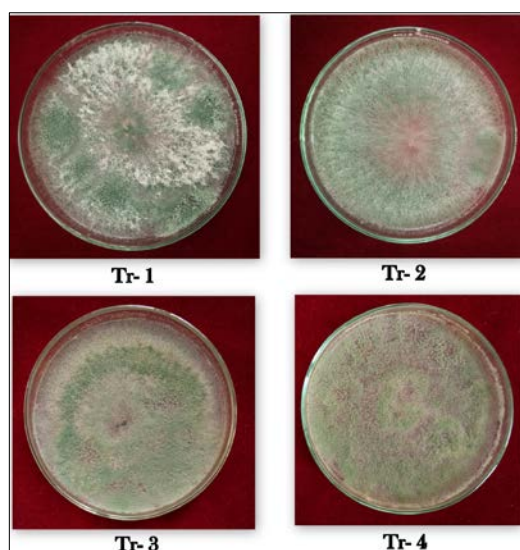


Fig 1: Morphological colonies of *Trichoderma* spp.

The present results are in agreement with [6], who have isolated *Trichoderma* spp. and studied the colony colour as velvetinous with white and dark green floccose surface along with scattered green patches and yellow to green pigmentation on PDA medium.

The effect of different *T. harzianum* formulations on two chilli cultivars i.e. Pusa Sadabahar and Navjyoti [7]. *T. harzianum* suppressed the symptoms expressions, conidial germination and 100% mycelial growth of *C. capsici*. A number of researchers reported the antagonistic ability of *Trichoderma* isolates against *C. capsici* are confirmation with the reports [8].

The four isolates of *Trichoderma* spp. and fungicide were screened against *Colletotrichum capsici* by dual culture and poison food test for their antagonistic and fungicide ability. Maximum inhibition growth was recorded in T₅ – Vitavax powder (82.20%) (treated check), followed by T₁ - *Trichoderma* spp. isolate 1 (76.45%) compared to the control represented in Table 3 and Figure 2,3,4 and 5.

Table 3: Efficacy of *Trichoderma* spp. isolates and fungicide against *Colletotrichum capsici* by dual culture technique and poisoned food technique

Tr no.	<i>Trichoderma</i> isolates	Mycelial growth of the pathogen (mm)	Per cent growth inhibition
T ₀	Contol	74.33	-
T ₁	<i>T. viride</i> isolate 1	17.5	76.45
T ₂	<i>T. harzianum</i> isolate 2	18.5	75.11
T ₃	<i>T. viride</i> isolate 3	28.5	61.66
T ₄	<i>T. harzianum</i> isolate 4	26.66	64.13
T ₅	Vitavax Powder	11.00	85.20
	S.Em±	0.58	-
	C.D. (at 0.05%)	1.305	-

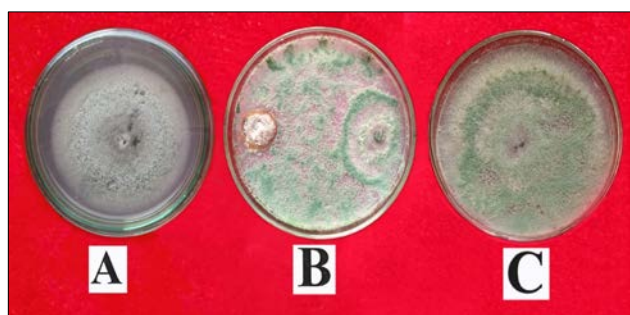


Fig 2: Efficacy of *Trichoderma viride* isolate 1 against *Colletotrichum capsici* by dual culture technique
A-Control of pathogen, B –Antagonistic effect of bio-agent over pathogen, C – Control of bio-agent



Fig 4: Efficacy of *Trichoderma viride* isolate 3 against *Colletotrichum capsici* by dual culture technique
A-Control of pathogen, B –Antagonistic effect of bio-agent over pathogen, C – Control of bio-agent

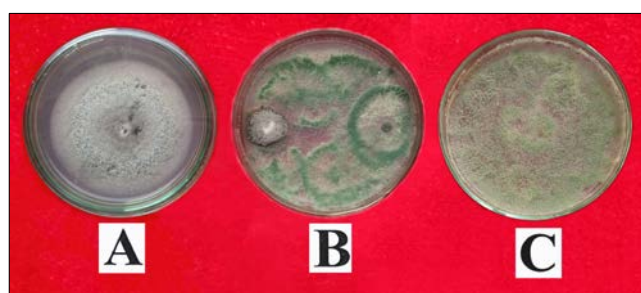


Fig 3: Efficacy of *Trichoderma harzianum* isolate 2 against *Colletotrichum capsici* by dual culture technique
A-Control of pathogen, B –Antagonistic effect of bio-agent over pathogen, C – Control of bio-agent

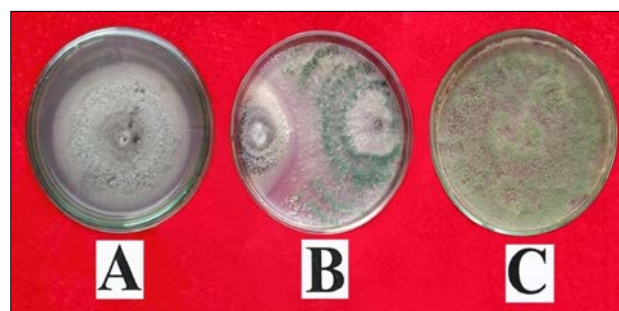


Fig 5: Efficacy of *Trichoderma viride* isolate 4 against *Colletotrichum capsici* by dual culture technique
A-Control of pathogen, B –Antagonistic effect of bio-agent over pathogen, C – Control of bio-agent

Minimum disease intensity (%) was recorded with Carboxin + Thiram (30.11%) which was used to compare as a treated check followed by *T. viride* + *T. harzianum* (Seed + Soil treatment) (36.33%) at 45, 60 and 75 DAT as compared to control respectively (65.69%) in Table 4.

It was observed that maximum plant height was recorded with Carboxin + Thiram (57.26 cm) which was used to compare as

a treated check followed by *T.viride* + *T. harzianum* (Seed + Soil treatment) (53.60 cm) at 30, 45 and 60 DAT as compared to control respectively (42.26 cm) in Table 4.

The effect of seed and soil treatment with *Trichoderma harzianum* and *Trichoderma viride* were significantly effective against seed borne fungal pathogens including *Colletotrichum* spp^[9]. Similar findings were also seen^[10, 11].

Table 4: Effect of *Trichoderma* isolates on Plant height and Disease intensity of chilli

Tr. No.	Treatments	Plant Height (cm)			Percent disease intensity (PDI)		
		30 DAT	45 DAT	60 DAT	45 DAT	60 DAT	75 DAT
T ₀	Control	15.4	34.66	42.26	29.14	50.08	65.69
T ₁	<i>T. viride</i> @ 4g/kg. Seed Treatment.	15.66	38.73	48.6	20.21	39.58	57.45
T ₂	<i>T. viride</i> @ 4g/kg Seed + Soil Treatment. @ 5kg/ha	17.13	40.8	50.2	16.03	29.71	48.23
T ₃	<i>T.harzianum</i> @ 8g/kg Seed Treatment.	16.73	40.2	49.53	18.01	32.63	55.01
T ₄	<i>T.harzianum</i> @ 8g/kg Seed +soil treatment @ 5kg/ha	19.06	43.46	52.46	13.59	23.53	38.66
T ₅	<i>T.viride</i> @ 4g/kg+ <i>T.harzianum</i> @ 8g/kg. Seed Treatment.	18.13	39.6	51.93	16.57	28.10	45.11
T ₆	<i>T.viride</i> @ 4 kg/ha+ <i>T.harzianum</i> @ 6g/kg. Seed + Soil Treatment @ 5 kg/ha.	19.2	45.06	53.6	12.36	22.22	36.33
T ₇	Carboxin 37.5% + Thiram 37.5% (Vitavax power 50 WP) @ 0.1% Foliar Spray.	19.66	47.2	55.46	10.38	18.45	30.11
	S.E. ±	0.48	1.88	0.52	3.00	2.52	2.23
	C.D. (at 0.05%)	1.03	2.94	1.27	1.72	1.44	1.25

Trichoderma spp. were found to be potential agents for the bio control which suppressed plant disease by protecting the plant from fungal infection. They are known to enhance plant growth promotion and reduce severity of many fungal diseases.

4. Conclusions

It was observed that the combination of *T.viride* and *T.harzianum* (seed and soil treatment) was proved to be most effective under field conditions when compared to the Vitavax powder 0.1% and the untreated control. *T.viride* isolate1 was most effective *in vitro* conditions followed by the other different *Trichoderma* isolates.

There fore it concluded that *Trichoderma* spp. can become a part in integrated management of Anthracnose of chilli.

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